Author’s response to reviews

Title: Early detection of Doxorubicin myocardial injury by Ultrasonic Tissue Characterization in an experimental animal model.

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Author’s response to reviews: see over
Cover Letter

Cardiovascular ultrasound

Reviewer, Dr. Maurizio Galderisi

We greatly appreciate your comments and suggestions. Your specific comments are listed below.

1- It is true that ejection fraction can detect the effect of DXB, but it was not able to detect doxorubicin myocardium injury in the lower doses infusion, where, at this time, an already significant change in ultrasonic tissue characterization indices was detected, suggesting that UTC can detect earlier signs of myocardium lesion in this model. From a clinical point of view, an early detection of myocardium dysfunction can probably indicate that a closer observation or modification of the treatment is mandatory.

2- We agree about the lack of diastolic function parameters. There are some limitations in the ultrasound technique to collect a series of well established parameters of diastolic dysfunction evaluation but a limited acoustic window was present and limited our options. An open chest model would be impractical. If we have used only E and A mitral flow curves only as parameters, we could have found some confusing data and we preferred to not include this analysis in the protocol. We included this limitation in the appropriate section.

3- We encountered little limitations of UTC in the model, but the Discussion section was rewritten in order to address this issue.

Reviewer, Dr. Gillian Whalley

We read your comments and deeply appreciate them. We modified the manuscript in several points according to them and we found that it was greatly improved. Below we reply your specific points.

1- In our laboratory one of the authors has extensively worked in this rat model using a sectorial transducer S12. It was also used by others to image rat hearts (Xiang et al. Cancer Chemother Pharmacol 2009;63:343-349.; Schwarz et al. Cardiovasc Res 1998;39:216-223.;Flynn et al. Stem cell research and Therapy 2012;3:36) without limitations in many fields of investigation. We did not have any problems with image quality specially in our sample of animals weighting more than 250g and some of them achieving up to 400g. All the technical settings for UTC were adequately done including the use of a rubber phantom to correct the IBS.

2- We disagree with the assumption that doxorubicin myocardium injury is well characterized. Besides extensively studied, there are still doubts about its major
mechanism of myocardial damage and it’s wide spectrum of time installation is an important clinical challenge. All available clinical tools use LVEF and we propose a new one capable of an early diagnosis of LV dysfunction. Nowadays, the identification of the left ventricle global failure occurs when it’s already installed and irreversible. Maybe and early identification would permit prevention of clinical dysfunction or, at least, postpone the deterioration by modifying the treatment.

Major points

1- Ejection fraction calculated by M Mode technique is accepted in experimental models of left ventricle failure due to diffuse myocardium commitment. M Mode images were obtained in a sweep speed of 100-150 mm/s what seems to have an adequate frame rate to an animal with a heart rate of 300bpm. Below we provide an example of M Mode image from the study protocol. An explanation of it was included in the methodology to clarify this point.

2- To correct the effect of repeated measurements and then, to avoid the problem of repeated individual measurements we calculated the beta coefficient of the linear regression analysis of each animal in subsequent dosages of doxorubicin infusion. After this, the beta coefficient was compared between groups, and this analysis is included in the Results Section. An example of this is presented below:
\( \beta \) = coefficient obtained from the linear regression of each parameter measure through different dosages of doxorubicin infusion

**Minor points**

1- The pathological analyses were not done to rats that died spontaneously and we corrected the text in order to avoid this misinterpretation. The animals were sacrificed after anesthetized, like was planned in our project. All spontaneous deaths were excluded as defined in the experimental protocol, although they may present more aggressive lesions.

2- The formatting errors pointed were corrected in the text and a full revision was performed.

SAL: animal submitted to saline infusion